Workshop on recommendations for pharmaceutical companies regarding data required for new antituberculous drugs

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Participants:

Gunnar Kahlmeter  Växjö, Sweden  EUCAST
Rafael Cantón  Madrid, Spain  EUCAST
Derek Brown  Peterborough, UK  EUCAST
Christian Giske  Stockholm, Sweden  EUCAST
Johan Mouton  Rotterdam, The Netherlands  EUCAST
Sören Gatermann  Bochum, Germany  EUCAST
Ron Jones  North Liberty, USA  EUCAST
Emmanuelle Cambau  Paris, France  ESGMYC
Nicolas Veziris  Paris, France  ESGMYC
Vincent Jarlier  Paris, France  ESGMYC
Miguel Santin  Barcelona, Spain  ESGMYC
Miguel Viveiros  Lisbon, Portugal  ESGMYC
Radu Botgros  London, UK  EMA

Some of the discussion related to confidential information supplied to EUCAST by pharmaceutical companies. Confidentiality agreements with the companies have been signed by all those attending the workshop.

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Presentations:

1. EUCAST approach for new agents. **Rafael Cantón**

**Summary**
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has the remit to establish clinical breakpoints, to set epidemiological (microbiological) breakpoints, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies, (including the European Medicines Agency, EMA), and European networks involved with antimicrobial resistance and resistance surveillance.

Most of the clinical breakpoints that are published annually on the EUCAST website ([www.eucast.org](http://www.eucast.org)) were established as a consequence of a process of harmonization of breakpoints that existed in Europe before EUCAST was established. Although this process was essentially finished in 2010, some of the harmonized breakpoints are currently under review. For new agents, the EUCAST breakpoints are established during the marketing authorization process conducted by EMA and its Committee for Medical Products for Human Use (CHMP). The specific process of setting breakpoints for these new agents is explained in two “standard operation procedures” (SOPs) that define the mutual agreement of EMA and EUCAST and all information that is required for the procedure (1, 2). For setting clinical breakpoints, EUCAST request from pharmaceutical companies scientific and technical information on the antimicrobial agent that include:

i) structure, mode of action, resistance mechanisms and potential selection of resistance,

ii) clinical indications and expected benefits over existing agents,

iii) microbiological activity (full species-related MIC distributions, not MIC\(_{50/90}\)),

iv) administration forms and dosages,

v) pharmacokinetic data in healthy volunteers and patients,

vi) activity in animal models,

vii) pharmacodynamic studies including Monte Carlo simulations,

viii) clinical studies

ix) relationships between dose, MICs and clinical and microbiological outcomes.

All this information is presented by pharmaceutical companies during EUCAST Steering Committee (SC) meetings in which they also propose preliminary breakpoints and present a timescale for studies and regulatory approval. All comments and potential modifications of the breakpoints (if necessary) are summarized and included in the EUCAST SC meeting minutes. Questions from the EUCAST SC are sent with these minutes to the pharmaceutical company, which may reply and request an additional presentation to a subsequent EUCAST SC meeting. If necessary, the process is continued until a decision on breakpoints is taken. Finally, breakpoints are reported to EMA, which makes the decision on the Marketing Authorisation Application and, if marketing authorisation is granted, includes the breakpoints in the summary of product characteristics (SmPC).

Up to now, EUCAST has decided breakpoints for nine new agents, including two new antituberculous drugs, delamanid and bedaquiline. Breakpoints for these new antituberculous drugs will be included in the breakpoint tables, version 5.0. Other new antibacterial drugs are now under review and include one oxazolidinone, two cephalosporins plus beta-lactamase inhibitor combinations, one carbapenems plus beta-lactamase inhibitor and two glycopeptides. None of them were submitted to EMA with clinical indications related with mycobacteria.
References
1. SOP/H/3043 Harmonisation of European Breakpoints set by EMEA/CHMP and EUCAST, 23 January 2007

Discussion points
- There is a good relationship between EUCAST and EMA. During the marketing authorisation application (MAA) process for a new agent EUCAST gives recommendations on breakpoints and EMA is the official agency that makes a recommendation to the EU on the MAA.
- During the breakpoint setting process there are frequently several “back and forth” discussions between EUCAST and the product team from the company presenting a new drug. EMA is copied in on all communications.
- Dosage is one of the issues discussed with regard to the indicated use of the agent.
- Formal review of breakpoints, possibly resulting in revision, can be requested by the company or by EMA, or may be instigated by EUCAST either directly by the Steering Committee or on request from a National Antimicrobial Susceptibility Testing Committee (NAC). The review may be needed because of new dosages, formulations, clinical indications or target organisms for the agent, new clinical data or resistance mechanisms, a new agent in the same class or new PK/PD data.
- Breakpoints recommended for delamanid were S≤0.06 mg/L, R>0.06 mg/L and for bedaquiline S≤0.25 mg/L, R>0.25 mg/L.
- Radu Bogros noted that the EMA Standard Operating Procedure (SOP) defining the relationship between EMA and EUCAST is being revised. Also a concept paper on revision of the EMA addendum to the note for guidance on evaluation of medicinal products indicated for treatment of bacterial infections to specifically address the clinical development of new agents to treat disease due to Mycobacterium tuberculosis has just been released for discussion (for comment by February 2015).

2. The current regulatory approach. Differences between antibacterial agents and antituberculous agents Radu Botgros

Summary
The Addendum to the Note for guidance on evaluation of medicinal products indicated for treatment of bacterial infections to address the clinical development of new agents to treat Mycobacterium tuberculosis (MTb) disease (EMA/CHMP/EWP/14377/2008) was developed during the period 2008-2010, at a time when new anti-TB candidate medicines were proposed mainly for inclusion in shortened regimens to treat drug susceptible (DS) tuberculosis or for addition to optimised background regimens for treatment of drug resistant (DR) tuberculosis. This Addendum came into force 1 August 2010 and still applies.

The document focuses on the evaluation of a single new agent within regimens that contain authorised anti-tuberculosis agents and gives guidance over non-clinical and clinical aspects relevant for the development of anti-TB medicines.

Guidance on the on non-clinical (in vitro and in vivo) evaluation of the potential efficacy of the candidate medicines is given, while existing limitations (the lack of a perfect animal model to predict clinical efficacy, the less advanced PK/PD techniques as compared with other antibacterial agents) are recognised. Indication regarding the extent of exploratory clinical studies needed before selecting one or a few test combination regimens to be evaluated in confirmatory studies of efficacy is also given.
The document addresses the selection criteria based on which patients are enrolled in clinical studies so that the possibility that the results of susceptibility testing confirm their eligibility is maximised and touches upon the importance of stratification according to important baseline factors. It is stated that extrapolation of treatment efficacy between DS and DR MTb is not possible, hence the need for separate appropriate clinical studies for each of the two situations. The document recognises that exploratory clinical studies of efficacy may allow for the selection of regimens for further studies based on biomarkers, but recognises that none of the existing biomarkers at the time when the Addendum was developed was shown to predict clinical outcomes at 24 months post-therapy.

Confirmatory studies may assess the efficacy and safety of a candidate medicine in a number of scenarios, e.g.:

a) the addition to/substitution for another agent within standard regimens to achieve either a shortened regimen, an improved safety profile, a lower potential for drug-drug interactions or a simplification of the DS MTb treatment or

b) the addition to optimised background treatment (OBT) regimens based on susceptibility test results to achieve superior efficacy to placebo + OBT in the treatment of DR MTb.

These confirmatory studies should follow-up patients for 24 months post-therapy.

Guidance is given on paediatric TB, where extrapolation of efficacy (and to a certain extent also safety) from adults to some paediatric age groups may be justifiable, provided that appropriate age-specific dose regimens based on PK data obtained in children with TB are established.

The fact that the evaluation of the safety profile of a TB test agent is confounded by its administration as part of combination regimens in clinical studies is highlighted and the importance of a comprehensive risk management plan (RMP) is acknowledged.

It was recently recognised that due to (a) recent advances in the understanding of the predictability of biomarkers for clinical cure in TB relevant PK/PD-related techniques (hollow-fibre models), (b) emerging data on relationships between early sputum colony counting (SCC) and final outcomes, and not least (c) with the approval of new TB medicines (with implications for the likely success of a study seeking to demonstrate superiority of a new medicine over placebo when added to OBT), certain aspects of the document require updating.

The European Medicines Agency (EMA) Committee for Human Medicinal Products (CHMP) has therefore recently adopted a Concept Paper on the revision of the Addendum, to be shortly published on the EMA website for a 3-month public consultation period.

**Discussion points**
- More details were given on how EMA sets guidelines:
  - A concept paper is written by the CHMP Infectious Diseases Working party
  - The concept paper is released for public consultation
  - A reflection paper and draft guidance are subsequently prepared
  - The reflection paper and the draft guidance are released for consultation
  - Following revision based on the received comments the guidance is updated and presented to CHMP for approval
  - After approval, the guidance is published on the EMA website.
- Revision of the EMA conditional approval is done each year (annual renewal), and this includes the evaluation of potential safety and resistance issues.
• It was questioned whether using mono-resistant strains as a surrogate for extreme drug resistant (XDR) strains will be acceptable.

**Relevant EMA guidance**
Addendum for guidance on evaluation of medicinal products indicated for treatment of bacterial infections to specifically address the clinical development of new agents to treat disease due to *Mycobacterium tuberculosis*

EMA/CHMP/EWP/14377/2008

**Additional documents released by EMA after the workshop**
Concept paper on revision of the addendum

EMA/CHMP/644851/2014:

Form for submission of comments on the concept paper.
The deadline for comments is 28 February 2015

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3. Lessons from the delamanid and bedaquiline MAA documents submitted to EMA.

*Emmanuelle Cambau*

**Summary**
ESGMYC has participated in EUCAST discussions about the setting of breakpoints for the two new antituberculous agents (ATTB) that have been submitted to EMA from 2011 to 2014: delamanid (DLM) and bedaquiline (BDQ). The objective of the presentation is a critical analysis of the microbiological data presented by the companies on the activity of their new drug against *Mycobacterium tuberculosis*, and especially in the field of multi-drug resistance. This is of particular importance since this is the first time EUCAST had to analyse data on antimycobacterial agents and this has been about 50 years since the last ATTB was released into the market.

From the data presented by the company, we may see that investigation of the mode of action and mechanisms of resistance are usually incomplete since first, they can work mostly on in vitro selected mutants, and second, it is known from already used ATTB that clinically resistant strains are frequently different from in vitro mutants. Globally, selection of resistant mutants for DLM is on the same range as isoniazid and for BDQ is as rifampicin. DLM targets the mycolic acids synthesis but requires cell transformation to be active while BDQ targets subunit C of ATP synthase. Cross resistance was not observed with other ATTB. MIC distribution for *M. tuberculosis* wild type strains was quite similar to other bacteria although different methods have been chosen by the companies. These methods were not always concordant and, consequently, this raised the problem of having breakpoints with regard to methods or to the medium used, which did not seem to be acceptable neither by EUCAST or ESGMYC. FOR BDQ, EUCAST and FDA gave concordant comments that one of the methods used, REMA (resazurin microtiter assay), was not a method validated to determine MIC and MIC determination had to be redone by another method. In addition, the MIC results did not show to be reproducible even for the quality-control strain used H37Rv and the reference method as agar dilution in 7H11. Finally, the results of susceptibility and resistance determined during clinical trials seem to be of low quality not only for the new drugs (large variation in MIC values) but also for the other ATTB (S then R, then S again!) to which the new drug is combined. This raises two problems: (i) the low quality of drug susceptibility testing for other ATTB and the DST method used during the trials, and (ii) the selection of the patients to be included since in some cases we observed an effective monotherapy with the new ATTB, the strain being resistant to all other ATTB. At the end, after the two new ATTB have been approved by EMA and release in to the market, we realized that the companies did not provide any SOP for routine individual
drug susceptibility testing, either for DLM or BDQ. This is now missing and we may think this should be included in the submission process, or determined by experts before the release.

The critical analysis showed how EUCAST and ESGMYC may work together for writing proposals to EMA and list the issues to be addressed.

Discussion points
- The importance of defining a reference method for MIC determination was highlighted.

4. MIC testing methods for *Mycobacterium tuberculosis* complex. Miguel Viveiros

**Summary**
An historical and technical overview on the currently available methods for, and concepts behind, determination of the susceptibility of *M. tuberculosis* to drugs and its possible use for the determination of minimum inhibitory concentrations was given. The Canetti’s and Middlebrook definitions of the critical concentration of antimicrobial drugs, the critical proportion and resistance were presented.

A short summary was presented on the solid medium, broth, direct and indirect culture methods that allow the execution of drug susceptibility tests and can be adapted to the determination of the MIC of drugs against *M. tuberculosis*. The Solid Medium, Middlebrook 7H10/7H11 agar medium, offers the most reliable and safe direct method for counting of viable drug resistant *M. tuberculosis* strains exposed to antmycobacterial drugs - a controlled preparation protocol does not inactivate by high temperature the drugs incorporated in comparison with the broth macrodilution method, which generates aerosols easily and needs added detergents to avoid clumping. The Lowenstein-Jensen medium is also a direct method of culture that can be used for DST and MIC determination but it is more difficult to count colonies and the control of its preparation and the incorporation of new antymycobacterial drugs is hampered by the partial inactivation of agents by high temperatures.

All other methods (MTT, REMA, MB/BacT, VersaTreK, and BACTEC (MGIT) use indirect methods to assess the viability of the cells exposed to drugs (oxygen consumption, pressure variation or oxidation of enzymatic substrates). The MTT and REMA tests are performed in 96 well microdilution plates and are very likely to produce aerosols. Also the reproducibility is very low due to many possible interferences, such as improper preparation, inaccurate dilutions, problems in preparation of inoculum and the huge metabolic plasticity of *M. tuberculosis* that allow many non-replicative cells to be metabolically active. The automatic broth culture systems (MB/BacT, VersaTreK, and BACTEC) are safe and very reproducible but have the disadvantage of high cost if many tubes or bottles are used, and all use indirect strategies to assess viability.

Noteworthy is the fact that Middlebrook 7H10/7H11 agar medium is recommended by the US regulatory agencies for drugs and medicines and is the method by which almost all the reference critical concentrations were calculated and determined in 1960-1970 for many of the anti-Tb drugs still in use. Lastly, a new method for semi-quantitative DST and MIC determination of drugs against *M. tuberculosis* has been developed for the Becton-Dickinson BACTEC 960 TB automatic broth culture system and this method was also presented and the rationale of the test detailed. Although it is a very reliable and reproducible alternative, it still is an indirect measure of viability.
Discussion points

- For a reference method, it seems important for all experts to use a direct method for reading growth on 7H11 medium (containing casein to enhance growth compared with growth on 7H10 medium) or macrodilution in 7H9 medium. It is also necessary to have a countable number of colonies on the agar (this should be monitored and care should be taken in preparation of the inoculum) and a reproducible method.
- Some new rapid methods, such as REMA and nitrate reductase, are not reproducible and cultures are easily contaminated.
- There is a need to be careful about the material used for tubes or plates, e.g. MB/BACT is not FDA approved.
- There is a need to look at the Global Laboratory Initiative recommendations from the WHO group (www.stoptb.org/wg/gli) and the ECDC Handbook “Mastering the basics of TB control – development of a handbook on TB diagnostic methods” (www.ecdc.europa.eu/en/publications).
- A good reference is the book Tuberculosis: Laboratory Diagnosis and Treatment Strategies (Advances in Molecular and Cellular Microbiology) edited by Timothy D Mc Hugh. CABI, 2013. It was suggested that it would be useful for this reference to be available on the website. It is unlikely that this would be possible but a link to the publisher could be included.

5. Wild type distributions for Mycobacterium tuberculosis complex with different testing methods. Gunnar Kahlmeter and Thomas Shön

Summary

A group of researchers with Thomas Schön as the principle investigator has for several years looked into wild type MIC distributions for M. tuberculosis (MTb). The studies have been performed with the following setup:

- Middlebrook 7H10
  - MIC WT-distributions for >90 unique isolates for most 1st/2nd line drugs
  - 96-well dispenser
  - Middlebrook 7H10 used in North America (CLSI-M24A, 2013)

- BACTEC 960 MGIT (Middlebrook 7H9)
  - >90% agreement within +/- one MIC dilution to 7H10 (1st and 2nd line)
  - BACTEC 960 MGIT widely used in clinical practice
  - Labour intensive and expensive for MIC
  - Alt. to MGIT: Commercial 96-well plates for testing ("broth-dilution MIC")

The Middlebrook medium is available both as agar and as broth. It is a synthetic medium but with additives like casein. There are several suppliers and manufacturers. BACTEC 960 MGIT is an AST apparatus produced by BD. It is used all over the world and has little competition.

Several MIC distributions were shown for the major first and second line drugs against MTb and in many of them strains with known resistance mutations were included. All distributions looked more or less as we have come to expect from other bacterial species.

During the meeting (TS was elsewhere engaged), via email communication, TS searched the Pubmed website for publications on MTb MICs for ofloxacin. The search criteria were: Mycobacterium tuberculosis ofloxacin MIC. This strategy resulted in 94 papers. A total of 1108 ofloxacin MICs were extracted from 11 studies where LJ or Middlebrook media were
used and a QC result was included. The MICs formed a perfectly typical MIC distribution where wild type and non-wild type were easily distinguishable and where a tentative ECOFF of 2 mg/L could be envisaged (see below) for ofloxacin, which also corresponds to the currently accepted “critical concentration”.

![MIC distribution for Ofloxacin](image)

A comparison of methods was also shown (see table).

**Comparison between methods**

- "In a comparison between MGIT and Middlebrook 7H10 medium of 7 first- and second line drugs including 133 MIC-determinations of 15 WT isolates we found an agreement of 91.7% within zone MIC-dilution step."

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<th>ECOFF</th>
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<th>WT</th>
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Schön, Kahntmeier et al. CMI in press. 2014

**Discussion points**

- In tuberculosis, one important task is to determine the activity of agents on “wild-type” strains. They could be strains from untreated patients.
- For wild-type distributions and ECOFF determination, genotypes are not looked at.
- The need to test strains from different geographical areas was discussed.
- An appropriate MIC method might include an inoculum adjusted to the density of a 0.5 McFarland standard and MIC defined as the concentration inhibiting 99% of the population.
- A quality control strain is needed and *M. tuberculosis* strain H37Rv is appropriate. MIC ranges for this QC strain should be established.
Stability of the drug should be addressed. There was discussion about the “real” concentration of the drug remaining active after long incubation. This might possibly be measured by examining the inhibitory effect on other known bacteria. This might work for drugs active against common bacteria such as *S. aureus* or *E. coli*, but not for specific anti-TB drugs.

- It was proposed that a set of 100 wild type strains should be assigned as reference strains and tested for different drugs. Twenty to 50 mono-resistant strains could be added to the set. This collection of reference strains would be shared between laboratories.

- There was a proposal to write an SOP on the reference method for MIC determination.


*Nicolas Veziris*

**Summary**

The basics of *Mycobacterium tuberculosis* susceptibility testing have been established in the 60’s. Georges Canetti and coworkers described a method that became the reference method for DST (1). The authors acknowledged that the determination of criteria of antibiotic susceptibility and resistance should ideally be based on clinical data since the main aim of these criteria is to indicate to the doctor if the drug will have its usual activity. However the activity of one drug against a strain that is not fully susceptible can’t be appreciated with confidence since tuberculosis is treated with two or three drugs. As a consequence the resistance criteria can only be determined according to bacteriological data. They also state that the concordance between bacterial resistance and clinical efficacy is another problem. The proportion method relies on the measure of the proportion of resistant mutants at a given concentration of antibiotic. The critical concentration (CC) is the one that is used in order to distinguish susceptible and resistant strains. The critical proportion (CP) is the minimum proportion of resistant colonies which must exist on the critical concentration so that the strain can be classified as resistant. The CP must be much higher than the average proportion of resistant mutants in wild-type strains in order to take into account the wild-type strains harboring high proportions of resistant mutants. The critical proportion must be a simple number easy to remember and easy to calculate. (1%, 10% and 100% were chose).

Regarding major antibiotics as isoniazid, the in vivo concentrations are much higher than the MIC. Hence if selection of resistant mutants occurs, it occurs at high level. They supposed that the proportion of strains resistant at low-level is low (although this can be discussed in regards of more recent data). Fixing drug resistance criteria at a high level opens the possibility that some resistant strains (low-level) be classified as susceptible. However the authors thought that “security” considerations should prevail in the choice of criteria. “Security” of resistance criteria is the aptitude to not categorize as “resistant” strains that are susceptible. The higher the criteria, the higher the security. The justification for this choice was the following: “It is a better choice to keep for isoniazid high resistance criteria so that the use of this admirable drug is never stopped too early”. Clearly the authors preferred missing low-level resistant strains than stopping isoniazid when it could still have some activity (even reduced). They considered that good resistance criteria had to satisfy one requirement which was to show the increase of frequency of drug resistant strains with the prolongation of treatment. That was shown comparing the proportion of patients harboring resistant strains related to the length of treatment.

Canetti and others met in 1961 and wrote the first international paper giving recommendations for DST (2). They retained the definition of resistance of the Canetti paper
based on laboratory response that is distinct from clinical response. They proposed CC and CP for isoniazid, PAS and streptomycin.

A new meeting of experts was held in 1968 and published the next year (3). The resistance definition that was adopted was the following: “Resistance is defined as a decrease in sensitivity of a sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild type strains of human type that have never come into contact with the drug”. They acknowledged once again that a diminished clinical response may occur when resistance is demonstrated but it is not certain. The security concept is recalled and should prevail for the choice of CC and CP. Criteria are adopted for new drugs: thioacetazone, ethionamide, kanamycin, cycloserine, viomycin, capreomycin, ethambutol pyrazinamide and rifampicin. Following this the Center for Disease Control proposed new CC that could be used in Middlebrook 7H10 or 7H11 media instead of Lowenstein-Jensen (4). The next step was the use of liquid cultures with the radiometric BACTEC 460 (5). Interestingly, when comparing 2 methods for DST with BACTEC 460 it appeared that the most efficient one was the one that used the same 1% CP as in solid media (6). Afterwards, the MGIT replaced the radiometric methods and was shown to be equivalent for DST (7). Currently there are CC that are proposed by WHO and by CLSI (8, 9). It must be underlined that CC change depending on the medium used for DST.

The interpretation is binomial for all DST techniques: susceptible or resistant. However recent data support the idea that instead of a unique CC there should be low and high CC being able to distinguish susceptible, resistant and intermediate strains (10-15).

References
Discussion points

- A quantitative determination of resistance might be preferred than only one critical concentration.
- What is the security side? to detect the low level resistance and not giving the drug, or giving the drug even a low level resistance in order the patient may have a benefit, even small.
- Caution is required since public expert documents (e.g. CLSI and WHO) may give different recommendations.
- It is not clear whether rifampicin should or should not be used in cases of low level rifampicin resistance.

Discussion session:
Moderators Vincent Jarlier and Miguel Santin

In the discussion session the following were agreed:

1. Reference methods for MIC determination
   There is a need for a “gold standard” method, which should be a direct method with visible growth.
   - The medium could be 7H10 or 7H11 (casein hydrolysate, calcium chloride)
   - The drug stability should be tested, e.g. by measuring the concentration at the end of incubation could be tested by using other bacteria (staphylococcus, Gram-negative bacilli or others) with MIC in the tested range; or with other methods (e.g. HPLC).
   - There should be at least three manufacturers of the recommended medium.
   - Media should be quality-controlled.
   - Reproducibility should be tested in different laboratories.
   - MIC distributions for new and for relevant comparator agents are needed with MICs determined by the reference method in different laboratories.
   - Growth should be determined after 21 days with a positive growth control.
   - An SOP should be written defining all details of the reference method (medium, inoculum, incubation atmosphere, incubation time, reading results, etc.).

2. Number of isolates to be tested
   - Working with MDR MTb should be avoided when possible as it may be dangerous.
   - A set of 100 wild type strains should be identified, i.e. strains lacking known mutations.
   - The group was convinced that, considering methodological difficulties, these MIC distributions will together form a very useful reference set of MIC distributions for MTb with appropriate agents.

3. Wild type and/or isolates resistant to other agents
   - In addition to the wild type strains, a set of 20–50 strains (more if possible) identified as resistant to one or two drugs but not MDR MTb. Each strain should have one or two mutations.
   - Some of the resistant strains may have cross-resistance to other drugs.
   - MICs should be determined by the same investigators and method as for the wild type strains. MIC distributions will be identified separately from that of the wild type strains.
   - There should be least 95% agreement between laboratories.
All laboratories should use the same quality control strains. If possible, some ATCC/NCTC/CCUG collection strains without and/or with known mutations will be identified – these can be used as QC strains and MIC reference ranges established in the future. A minimum of MTb H37Rv in duplicate should be included in each round of susceptibility testing for QC purposes in point 3 and 4 above.

4. How many laboratories involved in MIC studies and which laboratories (reference and/or routine)
   - At least three different laboratories should each determine MICs on the collection of wild type and resistant strains for a wide range of agents.
   - The laboratories should be reference laboratories in Europe.

5. Reproducibility studies
   - Ten strains, chosen to include some with MICs in the middle of the distribution and others with MICs at the edges of the WT distribution.
   - Each strain will be tested three times in three laboratories, i.e. nine determinations per strain.
   - Consequently, for these 10 strains, 90 determinations per strain will be gathered in order to calculate a median MIC. The range should be within two dilutions of the median MIC.

6. Studies of selection of resistant mutants in vitro
   - This may be useful but there was no time for discussion of that point at this meeting.

7. Relationship of in vitro results to clinical outcome
   - Need to apply the reference method defined above (point 1) to clinical isolates pre- and post-therapy during clinical trials.

Conclusions:
The group emphasized that identifying a reference method against which other methods can be calibrated is of utmost importance, not least for the development of new agents and for being able to give pharmaceutical companies clear instructions regarding what data must be brought to EUCAST for the process of setting breakpoints for MTb. There is still a need to discuss whether the liquid or the solid method should be the reference method.
   - The reference method will be used for the MIC studies and to consider this the reference method for agents submitted to EMA and EUCAST for breakpoints.
   - Some work is needed to determine a) how many manufacturers there are for the reference medium, b) what variation there is between manufacturers and between lots from the same manufacturer. It was believed that these tests could be performed with mycobacteria other than MTb, and the EUCAST Development Lab in Växjö, Sweden, with experience in these types of studies, volunteered to help.

Next steps:
   - Report on workshop to EUCAST Steering Committee, ESGMYC and ESCMID
   - Present conclusions at an open meeting. At ECCMID 2015 time will also be allocated at the General Committee meeting for this.
   - Comments should be sent to EMA on the concept paper before 28th February 2015.
   - SOP for the reference method will be drafted.
   - Selection of the reference strains will be started.
   - Reference laboratories will be asked to participate.

Notes compiled by Emmanuelle Cambau and Derek Brown